Free Radicals Produced in the Interaction of Cysteine with Carbonyls of Biological Relevance

PETER R. C. GASCOYNE, MARTYN C. R. SYMONS,*
JANE A. McLAUGHLIN, AND ALBERT SZENT-GYÖRGYI
Workshop of the National Foundation for Cancer Research, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, U.S.A.

Abstract

The free-radical-producing reactions between cysteine and the dicarbonyls glyoxal and methylglyoxal have been studied. Electron spin resonance data have allowed the radicals to be identified as complex substituted pyrazine cation radicals. A scheme for the production of these molecules is proposed, and a discussion of the relevance of such model reactions to biological charge-transfer processes is presented.

Introduction

It has been proposed that the electron transport essential to a variety of bioenergetic processes occurs by an intramolecular mechanism through the protein component of cellular structures [1]. Such transport, it is believed, may be facilitated by small molecules of high electron affinity which form local charge-transfer complexes with the structural proteins, lending to them the required physical reactivity [2].

A variety of quantum mechanical computations both on the energy-band structure of proteins [3] and on model charge-transfer interactions of dicarbonyls with simple amines [4,5] have been reported. These calculations verify the basic tenets of the intramolecular charge-transport theory, indicating that short-range, rather than long-range, electron transport within proteins is feasible [3], and that weak charge transfer between amines and carbonyls does indeed occur [4].

Experimentally the model charge acceptor methylglyoxal (2, oxopropanal) reacts with proteins to form colored products [6] with an increased electrical conductivity [7] and a small free radical content [8]. Amine models have been investigated in the hope that their fast tumbling in solution might permit electron spin resonance hyperfine couplings to be obtained together with corresponding structural information [9]. The radicals produced in these model reactions, however, appeared to arise not by direct electron transfer from amine to dicarbonyl, but rather after the occurrence of condensation reactions between the reagents. The radicals produced were most intense if the reagents chosen could

* Present address: Department of Chemistry, University of Leicester, Leicester, United Kingdom.

International Journal of Quantum Chemistry: Quantum Biology Symposium 9, 137–143 (1982)

CCC 0360-8832/82/010137-07$01.70

form hemiacetal, but not imine, linkages. For example, the SH-bearing molecule glutathione, which rapidly formed a hemimercaptal with the glyoxals studied, prevented imine linkage with amines, and thus acted to promote radical formation [9]. In this previous work no definite identification of the radical species was provided.

In the present paper the interaction of glyoxal and methylglyoxal with cysteine, which contains both the amine and sulfhydryl functions, is reported. For the first time in the study of the amine models the intense free radicals produced have been identified. A probable reaction pathway, and the nature of the radical-producing step, are considered. We also discuss the usefulness of such model systems for understanding biologically relevant charge-transfer interactions.

Experimental

Methylglyoxal (2, oxo-propanal) (40% aqueous solution, no. 17,733-4) and deuterium oxide (gold label, no. 15,188-2) were purchased from Aldrich Chemical Company; glyoxal (ethanedial) (40% aqueous solution, no. G-49) and diacetyl (diketobutane) (no. D-20) from Fisher Scientific Company; and L-cysteine (no. C-7755) from Sigma Chemical Company.

Individual aqueous solutions (0.4*M*) of cysteine, glyoxal, and methylglyoxal were prepared. Each solution was adjusted to pH 7.4 by dropwise addition of hydrogen chloride or sodium hydroxide. Similar solutions were also prepared using D_2O rather than H_2O. Equal volumes of cysteine solution and one of the dicarbonyl solutions were mixed, allowed to stand for 3 min, and then adjusted to pH 4.6 with sodium hydroxide. The mixtures were then transferred to a quartz-glass flat cell and investigated in a Varian E-109 electron spin resonance spectrometer. The mixtures were found to effectively deoxygenate themselves under these conditions, and there was no need, therefore, to degas them.

Similar experiments using diacetyl were performed using 0.1*M* solutions due to the poor miscibility of diacetyl with water. Simulations of the electron spin resonance spectra obtained in the various experiments were performed on a MacSym 2 computer system (Analog Devices, Westwood, MA) to confirm the correctness of the hyperfine coupling constants deduced from the experimental data.

Results and Discussion

The reaction of cysteine with glyoxal, methylglyoxal, or diacetyl proceeded with the formation of about 0.8 mol of acid per mole of cysteine. The pH of the reaction mixtures fell to about 3.4, and it was therefore adjusted with sodium hydroxide to the optimum value for radical formation (about pH 4.6). Under these conditions the reactions with glyoxal or methylglyoxal were found in each case to produce a single radical species. Visual observation of the reaction mixtures showed that a brown/green microprecipitate formed at the same time

as the most intense radical signal was apparent. A dark-green polymerlike mass eventually developed over a period of 36 h.

Deliberate addition of molecular oxygen to the mixtures was found to partially quench the radical signals. Attempts to initially degas the reagent solutions resulted only in a marginally stronger radical signal, again indicating that oxygen was not involved in the formation of the free radicals observed. No color or radical was observed in the reaction between cysteine and diacetyl, indicating that the aldehyde function was necessary for these aspects of the reaction. The free radical spectrum obtained from the glyoxal–cysteine reaction mixture is shown in Figure 1.

When D_2O was substituted for H_2O in the experiments, no change in the electron spin resonance spectra occurred, showing that no exchangeable protons contributed to the hyperfine or superhyperfine couplings evident in Figure 1. Analysis of the electron spin resonance spectrum of the glyoxal–cysteine reaction led to the identification of the nuclear hyperfine coupling constants shown in Table I. These constants allowed an accurate simulation of the observed radical species. Consideration of the possible chemical condensations which would allow for the exclusion of all exchangeable protons while giving rise to the observed hyperfine coupling constants led us to the conclusion that the radical produced was that shown in Figure 2. Development of this species is considered to have occurred first by formation of the thiazolidine compound in the manner proposed by Schauenstein et al. [10], and well known for the reaction of cysteine with formaldehyde [11] [Fig. 3(a), R = H]. After enolization [Fig. 3(b)] two such substituted thiazolidine molecules can undergo a straightforward condensation reaction to form either a polymer [Fig. 3(c), R = H] or the ring structure con-

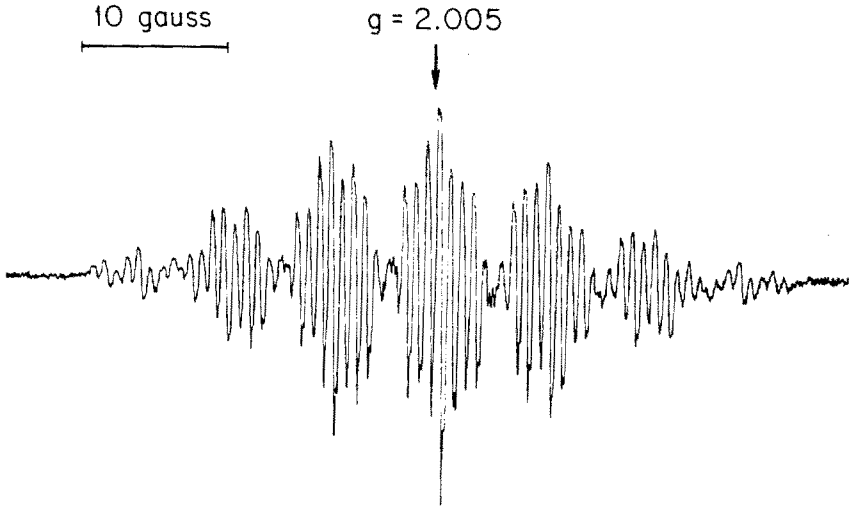

Figure 1. Free radical produced in the interaction of cysteine with glyoxal.

TABLE I. Hyperfine coupling constants (in gauss) deduced from the electron spin resonance spectrum shown in Figure 1.

Nuclear species	Number	Coupling Constant
Nitrogen	2	7.7
Proton	2	6.01
Proton	2	2.4
Proton	2	0.748
Proton	2	0.21

sidered to be the parent of the observed free radical [Fig. 3(d), R = H]. The dark-green precipitous mass which formed in the reaction under our mild conditions may have been the polymer shown in Figure 3(c) (R = H), although under much more rigorous conditions than our own the reaction of cysteine with methylglyoxal has been found to lead to a variety of degradation products [12].

The electron spin resonance spectrum obtained from the methylglyoxal-cysteine reaction was also clearly due to a single radical species. The signal was not as well resolved as in the glyoxal case, however, probably because of the introduction of the extra methyl hyperfine couplings which may have caused the apparent linewidth to increase, "blurring" the spectrum. Nevertheless, all aspects of the reaction were completely consistent with the glyoxal findings, and most probably the polymer and radical formed were also those shown in Figures

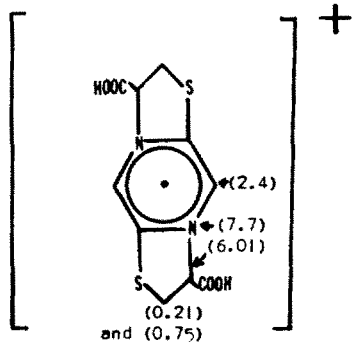

Figure 2. Cationic form (with neutral polar groups) of the free radical believed to give rise to the electron spin resonance spectrum shown in Figure 1. The assignments of the hyperfine coupling constants (in gauss) are shown in parentheses.

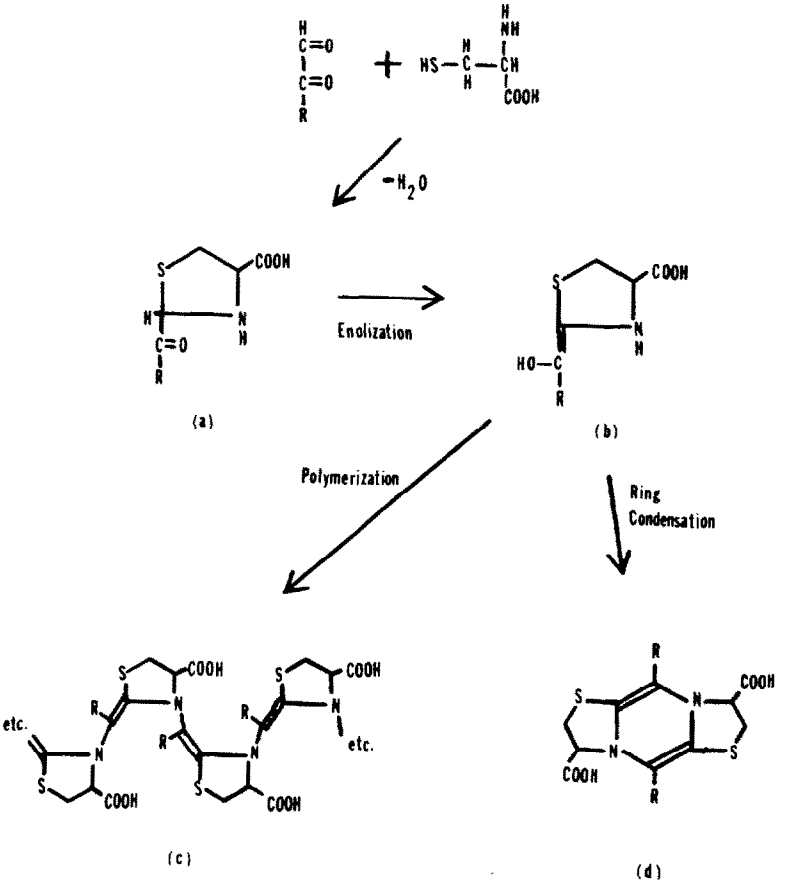

Figure 3. Pathway proposed for the reaction of glyoxal (R = H) or methylglyoxal (R = CH_3) with cysteine that leads to the formation of a polymer or the parent molecule of the radical shown in Figure 2.

2 and 3, with R = CH_3. In the final case of the reaction of diacetyl with cysteine, it may be noted that no aldehydic group was present for the formation of the thiazolidine ring, and even if such a ring formation were possible with a ketonic carbonyl, the enolization step [Fig. 3(b)] required for the further condensation was impossible because of the introduction of the nonlabile methyl group. These facts are consistent with the experimental observations that no dark products or radical signal formed in this reaction.

An interesting aspect of these reactions was the mechanism that produced the free radicals, since there is, apparently, no part of the chemical description which would suggest free-radical formation. This is also true of the previously reported reactions between glyoxal compounds and other amine models [9]. It is possible that the radicals were produced by direct electron transfer from the nitrogen-containing condensation products to the remaining unreacted dicar-

bonyls. Indeed, stronger radical signals were obtained when the reactions were performed in the presence of a small excess of the dicarbonyls. Analogs of the condensation products identified here, such as the pyrazines, have well-known electron-donating properties and, indeed, are substantially better electron donors than the simple amines used as reagents in our experiments. We can find no evidence in our present data, however, to confirm the formation of the semidione radicals that would be expected to result from such direct electron-transfer interactions (semidione electron spin resonance spectra have been presented elsewhere [13,14]). It would not be surprising, though, if such anion radicals were rapidly quenched by one of the many possible products in these model systems. Thus in the models studied to date, the only evidence for electron transfer directly to the simple dicarbonyls is circumstantial, and the electron-donating molecules are condensation products which are much better donors than the simple amines. This observation contrasts with the data for quinones for which electron transfer from simple amines appears to have been well established [15,16].

Finally, it appears from these considerations that the interactions of the glyoxal compounds with simple amines are not good models for the corresponding reactions with proteins due to the large steric effects present in the protein systems which evidently limit the degree of condensation that can occur. Ring condensation of the type found here for the model amines could only arise in protein systems if the dicarbonyls acted as crosslinking agents, such as has been observed for glutaraldehyde [17]. The circumstantial evidence presented here in support of electron transfer to the glyoxal compounds is nevertheless interesting and suggests that further work, perhaps with other biological charge acceptors such as quinones [18] and with better electron donors, must be pursued before a greater understanding of biological charge-transfer processes can be gained.

Acknowledgment

The authors would like to thank Dr. James Girard for helpful discussions.

Bibliography

[1] A. Szent-Györgyi, *The Living State and Cancer* (Dekker, New York, 1978).
[2] A. Szent-Györgyi, Int. J. Quantum Chem., Quantum Biol. Symp. **7**, 217 (1980).
[3] J. Ladik, S. Suhai, and M. Seel, Int. J. Quantum Chem., Quantum Biol. Symp. **5**, 35 (1978).
[4] P. Otto, S. Suhai, and J. Ladik, Int. J. Quantum Chem., Quantum Biol. Symp. **4**, 451 (1977).
[5] S. F. Abdulnur, Int. J. Quantum Chem., Quantum Biol. Symp. **4**, 217 (1977).
[6] J. A. McLaughlin, R. Pethig, and A. Szent-Györgyi, Proc. Natl. Acad. Sci. U.S.A. **77**, 949 (1980).
[7] R. Pethig, Int. J. Quatnum Chem., Quantum Biol. Symp. **5**, 159 (1978).
[8] P. R. C. Gascoyne, Int. J. Quantum Chem., Quantum Biol. Symp. **7**, 93 (1980).
[9] P. R. C. Gascoyne, Int. J. Quantum Chem., Quantum Biol. Symp. **8**, 265 (1981).

[10] E. Schauenstein, H. Esterbauer, and H. Zollner, *Aldehydes in Biological Systems* (Pion, London, 1977), p. 114.
[11] S. Ratner and H. T. Clarke, J. Am. Chem. Soc. **59,** 200 (1937).
[12] S. Kato, T. Kurata, and M. Fujimaki, Agr. Biol. Chem. **37,** 539 (1973).
[13] G. A. Russell and D. F. Lawson, J. Am. Chem. Soc. **94,** 1699 (1972).
[14] G. A. Russell, D. F. Lawson, H. L. Malkus, R. D. Stephens, G. R. Underwood, T. Takano, and V. Malatesta, J. Am. Chem. Soc. **96,** 5830 (1974).
[15] T. Yamaoka and S. Nagakura, Bull. Chem. Soc. Jpn. **44,** 1780 (1971).
[16] K. Muruyama, S. Suzue, and J. Osugi, Bull. Chem. Soc. Jpn. **44,** 1161 (1971).
[17] P. M. Hardy, G. J. Hughes, and H. N. Rydon, J. Chem. Soc., Perkin Trans. **1,** 2282 (1979).
[18] A. Szent-Györgyi, Int. J. Quantum Chem., Quantum Biol. Symp. **9,** 27 (1982).

Received April 2, 1982